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# Tests for Neuroplasticity in the Anuran Retinotectal System

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Mapping of retinotectal projections in the tree frog *Hyla regilla* was carried out by both behavioral and electrophysiological recording techniques following tectal ablations, with and without optic nerve regeneration. Scotomata produced by unilateral and bilateral half tectum ablations and by unilateral rectangular midtectal excisions were found to remain essentially unaltered in all cases through recovery periods up to 334 days. Similarly, electrophysiological mapping of the rostral half tectum separated by Gelfilm implants from the caudal tectum for up to 191 days yielded a normal rostral visual field. The stability of the retinotectal projection in adult *Hylidae* observed in these experiments contrasts with the plastic readjustments obtained in young goldfish in which the retinotectal system is still probably growing and presumably capable of field regulation. The results are taken to support the original explanatory model for developmental patterning of retinotectal connections in terms of selective cytochemical affinities between retinal and tectal neurons.

## INTRODUCTION

Regeneration of the optic nerve in lower vertebrates after section and mechanical scrambling of fiber relations is followed by an orderly reestablishment of the normal topographic retinotectal projection (3, 6, 21, 25, 26, 28, 31). If a substantial sector of the retina is removed, the remaining optic nerve fibers regenerate selectively to those tectal positions to which they normally connect (2). To explain this and other cases of selective reinnervation, Sperry proposed that during development the cells of the retina and tectum acquire individual position-dependent cytochemical specificities, which determine optic fiber outgrowth patterns and enable central synaptic connections to be formed in topographic order on the

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basis of preferential chemical affinities between matching retinal and tectal loci. It was suggested that the cytochemical labeling of individual neurons is achieved through a polarized field type differentiation of the retina and tectum separately along the three main embryonic axes (27, 29). Most of the subsequent work on the formation of retinotectal connections in amphibians, fishes, and birds (4) has supported this interpretation.

In apparent conflict with the foregoing, it has been found by Gaze and coworkers (9, 34) that the projection from each half of the retina of a surgically formed double-nasal or double-temporal composite eye in amphibians may expand over the entire tectum. Other studies involving ablation of the caudal tectum, transectal lesions, and Gelfim implants in goldfish have shown that the projection of the entire retina may become compressed onto the rostral half tectum and may reexpand with absorption of the film (12, 36, 37). It has been shown further by Horder (13) and Yoon (38) that a remaining hemiretina after retinal excisions may eventually expand its projection in time to cover an entire intact tectum or an entire inappropriate half tectum formed by a caudal hemitectal ablation.

In the light of these and related manifestations of plasticity in the retinotectal map it has been argued that Sperry's original interpretation is inadequate (7, 10, 12, 16, 23, 34) and Gaze (7, 10) has proposed a modified hypothesis in which the retinal fibers as a system complete for relative positions along the tectal gradients regardless of specific values within the gradient. However, all the foregoing experiments indicating synaptic plasticity have been carried out on embryonic anurans or on rapidly growing goldfish, where embronic type regulative processes might still be operative and thus account for the observed readjustments. In terms of the original model, the synaptic plasticity effects may mean merely that the position-dependent cytochemical tags are still labile and the local sign properties are simply reorganized as a developmental morphogenetic field following the imposed ablations, implants, and transplantations. Although this latter interpretation has been long favored in this laboratory (32, 37), critical experiments have not been advanced to distinguish between this and the alternative model proposed by Gaze.

In attempts to find a critical test between these alternatives, we have carried out half-tectum ablations and related interventions, where developmental regulation should be less likely to occur and where synaptic reconnection can be evaluated not only with electrophysiological but also behavioral methods. Postmetamorphic tree frogs, *Hyla regilla,* were selected because their tectal development is largely complete by metamorphosis (19, 33), and, hence, the tectal cytochemical tags might be expected to be more stable than in 5–11 cm goldfish, where the tectum is apparently still growing (18). The *Hylidae* are also highly visual, have

been found to show good optic nerve regeneration (*26, 27*) and are further well suited for behavioral perimetry testing as well as for electrophysiological recording.

Given stabilized tectal specificities, a lasting behavioral hemianopia would be predicted to follow hemitectal ablations according to the original explanation with no compression of the visual field measured electrophysiologically. If, on the other hand, nerve connections are formed according to the hypothesis of Gaze (*7*), there outght to be no behavioral hemianopia after recovery and electrophysiological mapping should show a compression of the whole visual field projection onto the remaining half tectum. The present findings support the first alternative, and it is shown how our original interpretation could account also for the various examples of plasticity that have been observed to date. A preliminary account of these findings has been given by Meyer (*22*).

## MATERIALS AND METHODS

*Animals.* Adult Pacific tree frogs, *Hyla regilla,* 30–40 mm in body length, were used throughout. They were housed in groups of three to six in small glass terraria of 0.5 cu ft at room temperature. Unlike most larger anurans, *H. regilla* and related *Hylidae* possess a cartilagenous cranium that is easily opened and closed for surgery and electrophysiology. Unlike the clawed toad, and more than the goldfish, these *Hylidae* are highly visual predators. The large optic tectum and easily obtained and large evoked tectal potentials are advantageous for tectal mapping. In addition, these frogs tame quite readily and maintain aggressive feeding behavior in captivity, qualities that proved important for the behavioral tests.

*Surgical Procedure.* Anesthesia was administered by placing the animal in an etherized atmosphere within a glass container for several minutes prior to surgery. This was sometimes repeated during surgery if indicated. All operations were performed through a dissecting microscope with variable magnification and coaxial illumination. The optic nerve was severed with the frog on its back and its mouth held open with special retractors. The mucosa was incised exposing the optic nerve at the point where it enters the cranium, and the nerve was repeatedly crushed with sharpened jewelers forceps until a clear discontinuity within the intact optic sheath could be seen.

The tectal surgery was done with the frog in the prone position. After a skin flap was cut free and reflected, a flap of cartilagenous cranium over the midbrain was incised with a small knife made of razor blade and reflected back underneath the nearby skin. The dura was opened with forceps.

The tectal region to be ablated was first outlined by inserting fine electropolished tungsten needles down to the ventricle after which the

area was aspirated with a small glass pipet. Care was taken to preserve the blood supply to the remaining tectum and at the midline. Gelatin film was implanted by inserting a cut rectangular piece of sterile ophthalmic Gelfilm (made by Upjohn of purified specially treated gelatin) 50–100 $\mu$ thick into a transverse mediolateral incision, so that it extended from above the tectal surface down into the ventricle and from the midline to the lateral tectal border, where it remained without significant adsorption through the course of the experiments. The cranial and skin flaps were returned to their original positions.

*Behavioral Testing.* A calibrated glass hemisphere 23 cm in radius was used for the perimetry measurements. It remained freely movable, resting its concave surface down on a white topped testing table. The frog's head was centered under the half dome by sighting through a small peep hole at the dome's vertical apex onto thin cross wires stretched inside of the hemisphere about 210 mm below the apex. The 0–180° wire was positioned to bisect the head the head down the midline, while the 90–270° wire bisected the two eyes. Small black circular or rectangular lures up to 4° in size were found to be highly effective as stimuli. They were presented on the end of thin glass rods against a white background formed by a cardboard cylinder extending 50° upwards around the outside of the hemisphere. To avoid extraneous visual stimuli, the examiner worked from within the posterior field of the right eye which was blinded by an optic nerve crush. The lure was advanced from behind along the outside of the perimeter into the posterior field of the left eye.

On each trial, the lure was moved in stepwise manner from the caudal pole toward the rostral pole along any given 10° parallel from the 0° horizon to 50° and oscillated over 2–3° for about a second or two at every 10° meridian. At some point in the progression, this typically produced a clear orienting response involving a sudden turning of head and body. At each response, the lure was immediately withdrawn, and the position recorded. The frog was then either allowed to spontaneously reassume its normal sitting position or was stimulated to do so by feeding a small fruit fly attached to the end of a glass rod, whereupon the perimeter was realigned. To minimize habituation the lure path was frequently varied, and an intertrial interval of a minute or more was preserved. Flies were periodically offered as reinforcement, and testing was carried out in sessions spaced over several days. Frogs that were highly reliable responders were selected in advance for the experiments, discarding about one-third of the initial supply. Responses were collected at each tested parallel until at least five within 20° of the most posterior response were obtained. When responses beyond this area became frequent, or when they became obviously inconsistent, the testing session was terminated. Since responses

beyond this 20° mark could be rather arbitrarily obtained by overtesting and were apparently more a function of habituation than variability in the measuring procedure, they are not included in the data presented. The remaining visual area outlined by these posterior response points was taken as the experimental blind region.

*Electrophysiology.* The method of electrophysiological mapping was similar to that employed and described by Gaze (5). Under ether anesthesia the cranium over one tectum was opened, and the animal subquently immobilized with *d*-tubocurarine and pinned onto a small platform. The dorsal surface of the tectum was then photographed at 47 × through a micrometer grid which gave 180-$\mu$ tectal spacings. This provided a rectangular array of potential electrode positions that could be identified on the real tectum by the tectal pigmentation pattern. The eye ipsilateral to the exposed tectum was covered with a small plastic occluder, and the animal was then placed in the center of a modified Brombach perimeter (American Optical Company). The contralateral eye axis was aligned by placing the transilluminated image of the optic disc along the central axis of the perimeter as viewed by a small telescope. This gave an axis of alignment some 5–10° anterior and inferior of that utilized by Gaze but was found to be more convenient and more reliable for these animals. The perimeter's horizontal meridian was adjusted to coincide with the long axis of the frog's oval pupil. Platinum plated tungsten microelectrodes with tip diameters of 2–5 $\mu$ were inserted perpendicularly up to 250 $\mu$ into the tectum. They typically recorded multiple units evoked by black disc-shaped stimuli from 2° to 3° in diameter presented on a white background. Usually the excitatory receptive field was 5–15° in diameter with a 3–5° central area of maximal response. The recordings were confined to the dorsal half of the tectum, since the ventrolateral surface was not readily accessible to probing. Following mapping sessions, the animal was either allowed to recover for subsequent recording or was sacrificed for histology.

*Histology.* The frogs were anesthetized with ether vapor, and the cranium opened over both tecta. The head was then cut free and immediately immersed in a mixture of 18 parts 80% ethanol, 1 part formalin, and 1 part glacial acetic acid. After several hours, the brain was dissected free and placed in fresh fixative for 12–24 hrs, then embedded in paraffin, and serially sectioned at 15 $\mu$. Each brain was stained with cresyl violet or a modified Bodian protargol stain. The gelatin implants were left in place throughout the histology.

## RESULTS

*Behavioral Perimetry: Normal Controls.* The posterior contour of the visual field of the left eye was mapped behaviorally in three normal ani-

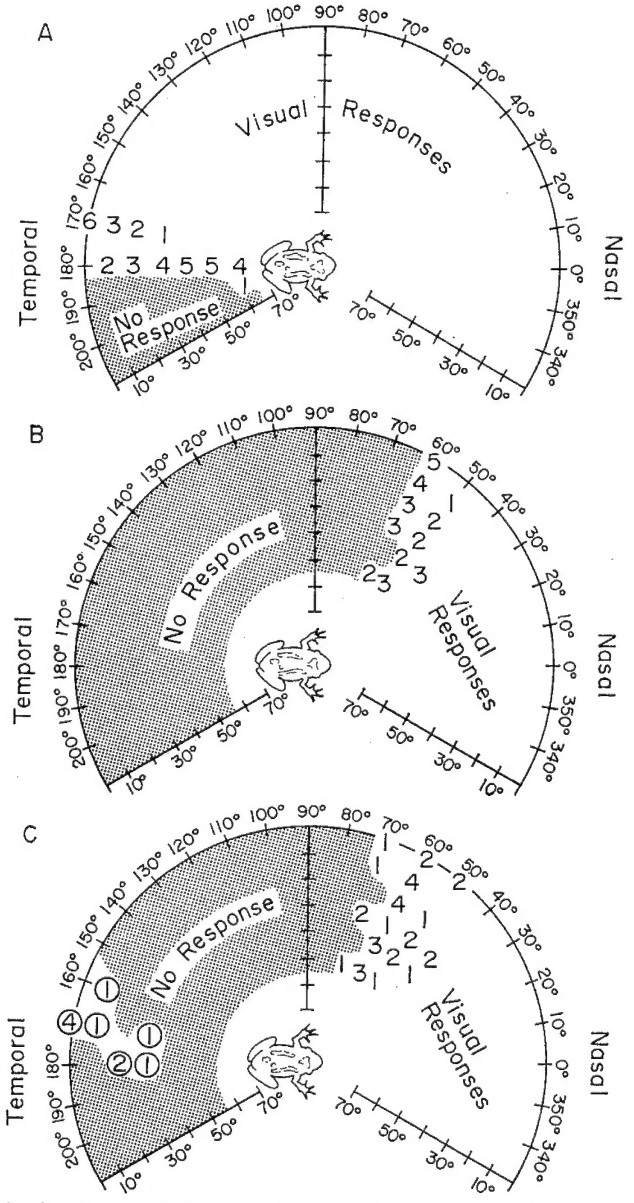


FIG. 1. Behaviorally obtained maps showing the posterior contour of left visual space. The stimulus was advanced in a temporal nasal direction along each tested 10° parallel until an orienting response was obtained. The number of responses at each position is shown, and the stippling indicates the visual field giving no responses. The right eye was blinded by nerve interruption in all cases. (A) Normal. (B) Frog with right caudal half tectum ablation sustained 7–10 days prior. (C) Same animal as in B, but tested after 124 days tectal surgery. Circled numbers represent contralateral orientation movements to the mirror image position.

mals. Testing was conducted between 4 and 5 days after the right optic nerve had been crushed, eliminating the right visual field on that side. Measured counterclockwise from 0° midline meridian, the left eye field was found to extend along the 0° parallel to 170°, along the 10° parallel to 180°, and along the higher parallels to 180–190°, depending on the individual animal (Fig. 1A). For each animal, the response points obtained at each parallel grouped quite tightly; often five or more responses were obtained at the same meridian, and none were separated by more than 10°. The posterior boundary of the visual field in different individuals differed less than 10° along any given parallel, suggesting a resolution of about 10° obtainable with this method. Since we are primarily concerned here with the presence or absence of a large half-field scotoma, accuracy of this mapping procedure is entirely adequate.

*Optic Nerve Regeneration: Tectum Intact.* Occasional responses could be obtained as early as 30 days postoperatively, but about 60 days were required for reestablishment of reliable responses throughout the whole visual field. The left visual field of three frogs was tested with the perimeter 110–122 days after crushing the left optic nerve and 34–48 days after recrushing the right optic nerve. It was clear that in each animal the regenerated map of the left visual field was normal in conformance with earlier reports (26, 27). Open field testing also confirmed this observation. Thus, with the tectum intact, regenerating fibers were found to reestablish the normal retinotectal map in this species as in other anurans.

*Caudal Half Tectum Ablation.* Behavioral perimetry testing of the left field was carried out in nine frogs which had the right caudal half tectum ablated with simultaneous crushing of the right optic nerve 6–9 days prior to testing. As expected (26), a large posterior scotoma was found in all animals (Fig. 1B). Typically the caudal posterior edge of the visual field fell approximately along the 60° meridian. Since the optic axis of the eye diverges from the midline by about 55–60° (8), this represents approximately a half-field scotoma. There was, however, some variation between animals. In one animal, the field bulged posteriorly as far as 100°. In another, the border fell along most of the 30° meridian and decreased to 10° along the 50° parallel. This variability in the maps is to be expected from individual differences in extent of the lesions and surgical trauma at the lesion border.

Between 130 and 133 days after the initial ablation, behavioral perimetry was carried out on four of the healthiest frogs to test for expansion of the visual field into the scotoma. The left optic nerve was not crushed because expansion appears to occur more rapidly under this condition (36). For testing purposes, the right nerve was crushed 14–15 days prior to the testing sessions. In three frogs, the border of the visual field was found to be not more than 10° different from that measured shortly after surgery

(Fig. 1C). In the fourth frog, some expansion up to 30° was found along the caudal edge. However, the original field of this animal was the smallest of the group and, as described above, extended temporally to only the 30° meridian. The initially enlarged scotoma could have resulted from reversible damage near the lesion that recovered to give a more typical half field size. In summary, the results in all four animals showed that the half-field scotoma had been preserved in essentially its original form up to 133 days postoperatively.

An incidental exception to the foregoing was observed; a false counter-reversal of responses aimed at the mirror image position in the visual field was obtained in all four frogs from the extreme posterior left field between the 160° and 180° meridians and between the 0° and 40° parallels (Fig. 1C). Such reversed orienting movements have been described (27) and can be attributed to the sprouting and regrowth of some of the optic fibers of the ablated tectal zone to the corresponding loci in the opposite tectum. The total area of the field giving incorrect responses was less than 30° of solid angle, and hence it would appear that only a small fraction of fibers was involved. Collateral sprouting from the ablated border or even at the chiasm could account for the result. The lack of any difference between the results from unilateral and bilateral tectal lesions described below indicates that this misdirected growth (not ruled out in goldfish) cannot be interpreted as having been responsible for the absence of field expansion.

*Electrophysiological Mapping: Normal Controls.* For reference purposes and comparison with previously published results, the left visual field was mapped on the right tectum in a total of 15 normal *H. regilla.* Good multiunit responses were easily obtained having excitatory receptive fields of 5–15° and a central maximal response region of 3–5°. The source of these action potentials is thought to be the terminal arborization of optic nerve axons (20) and probably reflects the pattern of functional connectivity between retina and tectum under normal conditions. All the maps were essentially the same and proved quite similar to those obtained from Rana and Bufo (5, 15).

A typical map is presented in Fig. 2A and is self-explanatory. The order of recordings did not seem to affect the map. Owing to the difficulty of recording from the undercurved caudal and lateral edges of the tectum, the actual extent of the tectal representation of the visual field is estimated to be larger than indicated by about 30° at the temporal extreme and 100° into the inferior field. This presents no particular difficulty for these experiments, since the experimental manipulations fall well within the field covered by these maps.

*Regeneration with Tectum Intact.* As expected from work on other species, *H. regilla* was found to regenerate the normal retinotectal pro-

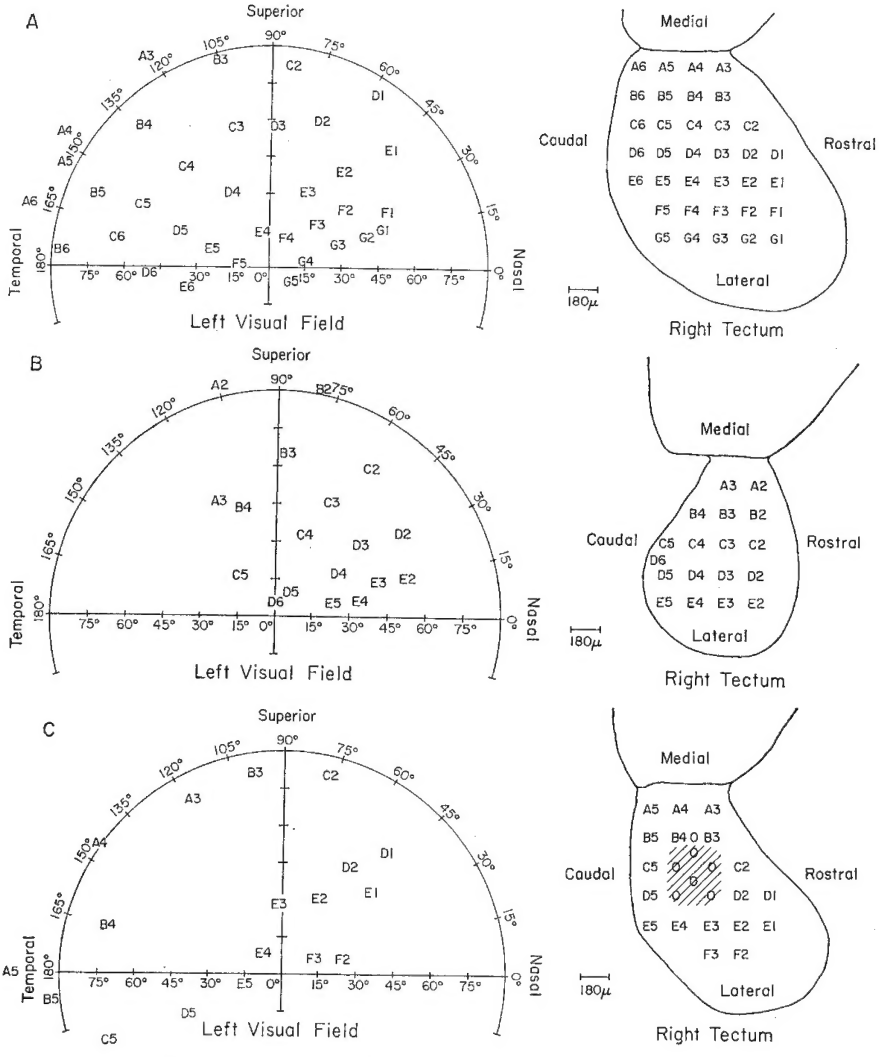


FIG. 2. Electrophysiological map of left visual space onto right tectum with numbers and letters indicating corresponding electrode placements and receptive field location. (A) Normal. (B) Right caudal half tectum ablation and left optic nerve crush 339 days prior. (C) Ablation of a rectangular piece of dorsal right tectum and left optic crush 106 days prior. Crosshatching indicates pia-covered ablation area with ○ indicating electrode positions giving no response.

jection after optic nerve interruption in the six cases tested between 60 and 151 days after complete axon interruption. Visually driven units could be recorded as early as 25–55 days in four other animals, but mapping

was not attempted because of low amplitudes and large receptive fields. The recording improved with time, and at 60–80 days multiunits with normal receptive fields located in the appropriate region of visual space could be obtained over the entire tectum. Consequently, mapping in these experiments was carried out subsequent to 60 days after nerve interruption.

*Unilateral Half Tectum Ablation.* Unilateral caudal tectal ablations produced the predicted electrophysiologically-measured scotoma at up to 2 weeks after surgery. This same half-field scotoma was found essentially unchanged for as long as 339 days. Excluding the seven animals used only for behavioral testing, fifteen frogs sustained right tectal ablations, of which ten of the healthiest survivors were selected for electrophysiological recording. In three of these, the left optic nerve was left intact, and retinotectal maps were obtained 114–147 days postoperatively. In the other six frogs, the left optic nerve was crushed within a day of tectal surgery and recordings obtained from 148–339 days after tectal ablation (Fig. 2B). There was no significant difference between the groups, and they will be treated together. A complete recording from the remaining animal was not obtained.

Temporal extent of the experimentally reduced visual field ranged from the 135° meridian to some 30–40° nasally. Despite probing near the lesion at close-spaced intervals, the temporal contour was found to be within 20–30° of that initially produced. The small changes sometimes found are most simply accounted for on the basis of reversible damage or differences in electrode probing. The field remaining was a well-structured fairly normal half field. There was no apparent difference between these and normals in the separation of receptive fields; whereas, a full field expansion ought to have produced interreceptive field distances about twice that of normals.

Minor departures from the normal field organization seen in four animals seem best explained by topographical distortion of the tectum, caused by its tendency to move gradually into the space created by the lesion. Thus, in nine animals with half-tectum ablation, the electrical mapping indicated a preservation of the original patterns of retinotectal connections.

In two additional *Hyla* with right tectal ablation and left optic nerve crush electrical recording was done at 35 and 37 days. As in 30-day regenerating normals, the units obtained were of low amplitude with diffuse, poorly ordered receptive fields suggesting the potentials were from exploratory fibers and fibers in passage rather than from finalized axon terminal arborizations. Among these were units in the mediocaudal right half tectum that had their receptive fields within the scotoma, indicating again that the lack of field expansion after nerve regeneration was not attributable to absence of the appropriate fibers.

*Bilateral Ablation of Caudal Tectum.* Bilateral half tectum ablations were performed in eight animals to deprive the disconnected fibers of alternative "correct" sites on an intact tectum and also to test the possibility that visual field expansion might be indirectly facilitated through chemical or other effects. Mapping of left visual space onto right tectum was performed in the first four operated animals between 60 and 106 days after bilateral optic nerve crush combined four days later with blilateral caudal tectal ablation. The resultant electrophysiological maps were the same as those obtained from animals with unilateral lesions.

*Rectangular Tectal Lesions.* Rectangular lesions similar to those reported to cause compression of retinal fibers in goldfish (23, 36) were placed in the right tectum of five frogs, and the left optic nerve was divided at the same time. The 600 × 800 $\mu$ tectal ablation was centered rostrocaudally with the 600 $\mu$ edge parallel to and about 100–200 $\mu$ from the midline. This spared much of the tectum, including a medial strip containing the medial optic tract.

Maps obtained from three of the four survivors between 76 and 171 days postoperatively were essentially normal with a large scotoma corresponding closely in dimension and location with placement of the lesion (Fig. 2C). No evidence was found for compression of fiber projection along either the rostrocaudal or the mediolateral axis. In the fourth animal, only six receptive fields could be mapped at this date, but none fell within the scotoma area.

*Gelatin Film Tectal Implants.* Mediolaterally-oriented gelatin film implants in the goldfish tectum induce the same kind of fiber compression that is produced by caudal lesions (37). Pieces of Gelfilm were inserted into the right tectum of nine animals, essentially cutting off the posterior from the anterior half of the tectum. Recording was carried out in two of these at 110 and 204 days postoperatively. In a third case, the left optic nerve was crushed at 114 days, and mapping was performed at 291 days after tectal surgery. In all three of these cases selected for postsurgical recording, mapping of the rostal half tectum yielded a half visual field entirely similar to that for the same region of a normal tectum. Histology in these three and five additional specimens with implants showed the implant to be intact in all cases, properly placed, and apparently not invaded or crossed by any nerve fibers.

*Histology.* The intact tectal tissue appeared normal and extended to the edge of the lesion. The tectal cytoarchitectonics seemed well preserved except at the very edge of the lesion. The tectal tissue tended to move gradually into the space created by the lesion, but the flat sheet-like tectal structure was preserved with no dorsal or ventral curling. Scar tissue appeared to be limited to the immediate vicinity of the lesion. There was

no indication of tectal cell regeneration nor of any abnormal growth covering the intact tissue. In short, the remaining tectum appeared to be healthy and normally accessible to microelectrode probing. This conclusion is based on examination of all surviving experimental animals, 15 with unilateral lesions, 8 with bilateral lesions, 9 with gelatin implants, and 2 with rectangular lesions. To optic fibers deprived of tectal connections could not be distinguished from nonoptic fibers in these preparations, and their fate remains to be determined.

## DISCUSSION

Behavioral perimetry testing following ablation of the caudal hemitectum showed a stabilized temporal half-field visual scotoma that remained essentially unchanged for 130–133 days. Similarly, electrophysiological mapping after unilateral and bilateral caudal tectal ablations, midtectal rectangular ablations, and mediolateral gelatin tectal implants all showed a corresponding stabilized scotoma with no significant compression for periods up to 339 days. The results were the same with and without optic nerve division and regeneration. These findings stand in direct contrast to those obtained in goldfish, where the initial scotoma produced by essentially the same lesions vanished within 90 days, correlated with compression of the total retinotectal projection map onto the remaining tectum.

The observed stability of the projection map in *H. regilla,* and probably also in *Xenopus,* according to preliminary evidence (7), appears to rule out the alternative hypothesis of competitive innervation and favors the original interpretation based on specific cytochemical affinities between retinal and tectal neurons. The various plasticity effects reported in goldfish can be explained on the assumption that the presumably actively growing goldfish tectum (18) still possess developmental lability in its neurospecificity properties. The surgically isolated tectal half field may be presumed to undergo reorganization into a new whole field bringing corresponding changes in its cytochemical local sign or "position-dependent" properties that determine fiber connections. This would be predicted for early intervention in any developing morphogenetic field by definition; it is only surpising that these cytochemical dynamics should remain labile so late in growth. The adult frog tectum by contrast appears to have completed growth by cell addition at metamorphosis (19, 33).

The original explanatory model (27, 29, 32) was expressed from the start in terms of the already established concepts of the embryonic morphogenetic field and/or graident (35), which by definition has plastic "systems" properties such that, if cut in two, either isolated half will form a whole. To impute a "rigid place specificity" (7, 9, 12, 34) or "rigid cell-to-cell connection" (10, 16, 23) reflects a misinterpretation of these basic

concepts in developmental biology. The retinal unit specificities were always inferred to be plastic and reversible with the dorsoventral axis retaining plasticity later than the rostrocaudal (27), and individual retinal fibers were described as typically connecting over a wide tectal area with considerable overlap among terminals of neighboring ganglion cells depending on the steepness of the retinal and tectal gradients (30, 31).

The findings for experimentally-formed composite eyes (9, 34) may be explained by supposing that each hemiretina of the composite eye reorganizes itself to differentiate into a complete retinal field (32, 38), with each twin field a mirror image of the other. Such twinning occurs also after similar surgical manipulations of limb buds (1). That the composite eye is in this and in other respects a double or twinned, rather than single, system is indicated in the tendency for such eyes to form double lenses (11).

Expansion of the tectal projection from a hemiretina to cover the entire tectum (13, 38) or a presumably reorganized half tectum (38) would be predicted for rapidly growing goldfish when sufficient time is allowed for the isolated half retinal field to reorganize itself into a whole field, especially where such reorganization is facilitated by destruction of the outer layers of the eyeball along with the retina (38). These were avoided in the early experiments showing selective tectal reinnervation (2, 17). Plastic mapping of tectal projection upon absorption of a dividing Gelfilm implant in goldfish (37) also is what our interpretation would predict upon restoration of cell contacts across the division line and resumption of intercellular exchange of field position information. This plasticity with respect to expansion or compression of a developing field should be distinguished from that involving reversal of polarity. The expectation that plasticity for a complete change of polarity of a gradient would be lost before that for mere expansion or compression seems to be borne out in early data (24, 39). Our interpretation hardly permits the designation of an "unspecified state" (14) for the retinal field, especially without reference to these two very different aspects of field dynamics. By our model, most of the experiments that have seemed to deal with a critical period for the specification process (16) have dealt instead with the period in which specificities already long established become irreversibly fixed, i.e., a given aspect of field plasticity can no longer be altered by a particular experimental manipulation. The initiation of retinal specification is assumed to begin in the early embryo and to emerge gradually out of the larger main axial gradients of the whole embryo (32). A molecular model is proposed by McMahon (personal communication), describing development and behavior of these morphogenetic fields in terms of cellular contacts regulating morphogenic substances.

The present findings, as well as those of all the other retinotectal experiments to date, seem to be most simply accounted for in terms of the original chemoaffinity hypothesis. If our interpretation is correct, most of the retinotectal experiments demonstrating neuroplasticity can be seen to deal, not so much with direct regulative factors in nerve growth and synaptic formation, as with variables in the organizational dynamics of the developing morphogenetic field of the eye and tectum.

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